HHS Public Access

Author manuscript

Nat Rev Neurosci. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

Nat Rev Neurosci. 2017 August ; 18(8): 459–469. doi:10.1038/nrn.2017.71.

Neural circuits underlying thirst and fluid homeostasis

Christopher A. Zimmerman, **David E. Leib**, and **Zachary A. Knight**

Department of Physiology, Kavli Institute for Fundamental Neuroscience, and Neuroscience Graduate Program, University of California, San Francisco, San Francisco, California 94158, USA

Abstract

Thirst motivates animals to find and consume water. More than forty years ago, a set of interconnected brain structures known as the lamina terminalis (LT) was identified that governs thirst. However, due to the anatomical complexity of these brain regions, the structure and dynamics of their underlying neural circuitry has remained obscure. Recently, the emergence of new tools for neural recording and manipulation has reinvigorated the study of this circuit and prompted reexamination of longstanding questions about the neural origins of thirst. Here we review these advances, discuss what they teach us about the control of drinking behavior, and outline the key questions that remain unanswered.

Introduction

Why do we feel thirsty? Early theories posited that thirst is the local sensation of dryness in the mouth and throat[1,2], but we now know that thirst is a homeostatic response to changes in the blood: increases in plasma osmolality[3-6] or decreases in plasma volume[7,8] or pressure[9] trigger the sensation of thirst, which motivates animals to find and consume water and thereby restore these parameters to their physiological set-points.

The key brain structure for the genesis of thirst is the lamina terminalis (LT), a group of three deep forebrain nuclei that coordinate the homeostatic response to fluid imbalance (described in more detail below). While the importance of the LT for the control of drinking has been appreciated for decades (reviewed by refs. 10-12), our understanding of the underlying circuit mechanisms remains limited. For example, we still do not know the identity of most of the cell types that reside in the LT; the dynamics of those cells during behavior; or the anatomical pathways by which they transmit information to other brain regions. This knowledge gap reflects, in part, the complexity of the LT, which contains a diversity of intermingled neural cell types distributed across three small nuclei. While these features have traditionally made the thirst circuit challenging to dissect, the recent application of genetically targeted techniques has led to renewed progress.

In this Progress article, we summarize our current understanding of the neural circuitry underlying thirst and drinking behavior in mammals. First, we briefly overview the well-established roles of the LT and various circulating hormones in the regulation of fluid

Correspondence to Z.A.K.zachary.knight@ucsf.edu.

Competing interests: The authors declare no competing interests.

balance. Second, we describe recent insights from the application of genetically targeted methods – including optogenetics, calcium imaging, and viral tracing – that have been used to study discrete elements of the thirst circuit and characterize their function, dynamics, and connectivity in freely behaving animals[13-21]. Last, we highlight some of the key questions that remain unanswered.

The lamina terminalis

Our modern understanding of the neural control of thirst originated with the discovery by Bengt Andersson in the 1950s that infusion of hypertonic saline into the anterior hypothalamus of goats stimulates intense drinking and water retention (antidiuresis)[22-24]. James Fitzsimons later discovered that infusion of the hormone angiotensin II (AngII) into the same area of rats also produces thirst[25,26]. Together, these experiments identified a small forebrain region (the LT) that monitors homeostatic signals of fluid balance (plasma osmolality and AngII) and translates these signals into appropriate counter-regulatory responses.

The LT is composed of three small, interconnected structures that lie adjacent (anterior and/or dorsal) to the third ventricle. Two of these structures – the subfornical organ (SFO) and organum vasculosum of the lamina terminalis (OVLT) – are circumventricular organs, meaning that they lie outside the blood-brain barrier and therefore have direct access to the circulation[27]. Information about fluid balance enters the LT primarily through specialized interoceptive neurons in the SFO and OVLT. Some of these interoceptive SFO/OVLT neurons are intrinsically osmosensitive, meaning that their firing rate increases in response to increases in the tonicity of the extracellular fluid[28-31], and many of these osmosensitive SFO/OVLT neurons are also activated by the hormone AngII[32-35]. Additionally, some SFO/OVLT neurons may receive ascending neural signals from peripheral blood pressure sensors (baroreceptors)[36,37]. Thus, SFO/OVLT neurons are poised to integrate signals about plasma osmolality, volume, and pressure and then use this information to control thirst. The third component of the LT is the median preoptic nucleus (MnPO), which cannot access the blood directly and is thought to be an integratory center[38]. Together, these three structures form a forebrain hub for the regulation of fluid balance.

Signals detected in the SFO and OVLT are shared with each other and the MnPO through an extensive network of bidirectional projections[39-43]. Activation of this network then triggers a coordinated set of homeostatic responses that restores fluid balance. These responses include: behavioral mechanisms that motivate water and sodium consumption (i.e., thirst and salt appetite)[44-47]; autonomic mechanisms that modulate sympathetic outflow and thereby alter blood pressure and heart rate[48,49]; and neuroendocrine mechanisms that modulate water and sodium retention by the kidneys[50,51]. These neuroendocrine responses are mediated primarily by the hormones vasopressin (AVP) and oxytocin (OXT), which are released from specialized posterior pituitary-projecting neurosecretory cells in the paraventricular hypothalamus (PVH) and supraoptic nucleus (SON) that are under direct control of ascending input from the LT (reviewed by refs. 52,53).

Distinct neural cell types

The LT is neurochemically heterogeneous, but how this molecular diversity maps onto functional populations of neurons has been unclear. Experiments using optogenetics and chemogenetics have begun to define functional subsets of neurons in the LT based on their genetic and anatomic features[13-16,21] (Figure 1, Table 1).

SFO

The SFO contains at least two molecularly distinct populations of neurons with opposing effects on drinking behavior: a glutamatergic population (SFO^{GLUT} neurons) that promotes drinking[13-16] and moderate sodium consumption[15], and a GABAergic population (SFO^{GABA} neurons) that suppresses drinking[13]. SFO^{GLUT} neurons are activated *in vivo* by circulating signals that stimulate thirst, including increases in plasma osmolality and (via AngII) decreases in plasma volume or pressure[16]. Optogenetic silencing of SFO^{GLUT} neurons under these conditions suppresses drinking by thirsty mice[16], indicating that SFO^{GLUT} neuron activity is necessary for physiological drinking behavior. In contrast, the *in vivo* dynamics and necessity of SFO^{GABA} neurons are unknown. Dividing the SFO into glutamatergic and GABAergic subpopulations is almost certainly an oversimplification, but molecular markers that would enable more fine-grained distinctions have not yet been identified.

SFO neurons innervate several brain regions involved in fluid homeostasis[13,16,21], including the MnPO, OVLT, PVH, SON, and the ventrolateral part of the bed nucleus of the stria terminalis (BNSTvl), and SFO neurons can be further subdivided based on their projections to these structures. Optogenetic stimulation of $SFO^{GLUT}\rightarrow MnPO$[16] and $SFO^{GLUT}\rightarrow OVLT$[21] projections promotes thirst, whereas simultaneous optogenetic silencing of glutamatergic and GABAergic SFO→OVLT projection neurons suppresses drinking by thirsty mice[21]. This suggests that the MnPO and OVLT are the main targets by which SFO^{GLUT} neurons drive thirst.

Other SFO projections appear to mediate different functions. Optogenetic stimulation of the $SFO^{GLUT}\rightarrow$BNSTvl projection specifically increases sodium consumption[21], suggesting that this pathway is involved in salt appetite. Optogenetic stimulation of the $SFO^{GLUT}\rightarrow$PVH projection does not promote thirst[16], which is consistent with a primary role for the PVH in the neuroendocrine[53] and cardiovascular[54], rather than behavioral, responses to fluid imbalance. In contrast to their glutamatergic counterparts, SFO^{GABA} neurons innervate only the MnPO and OVLT[13,21], and the function of these projections has not yet been tested. Similarly, little is known about the microcircuits within the SFO that control drinking behavior, although there is some evidence that SFO^{GLUT} neurons receive inhibitory input from neighboring SFO^{GABA} neurons[16,21].

MnPO and OVLT

Relative to the SFO, cellular diversity in other regions of the LT has been less explored. One challenge is that the MnPO and OVLT are located in close proximity, making it difficult to selectively target neurons in only one of these structures by viral injection. Optogenetic stimulation of glutamatergic or GABAergic MnPO/OVLT neurons promotes or suppresses

drinking, respectively[17]. However, the broad extent of viral transduction in these experiments complicates their interpretation[17], and further progress will require identification of molecular markers for subsets of MnPO and OVLT neurons with specific functions.

The MnPO and OVLT share an overlapping set of projection targets with the SFO, including the SON, PVH, and BNSTvl, as well as reciprocal connections within the LT[39,41,43]. These projections have yet to be functionally annotated or mapped with cell type-specificity. Given the high degree of interconnectedness between structures in the LT, it will be critical to dissect the degree of collateralization, redundancy, and necessity of efferents from each region with cell type-specificity in order to fully understand how information flows through the thirst circuit.

Anticipatory signals

Neurons in the LT play a well-established role in monitoring the state of the blood, but whether these cells receive other types of signals that control drinking has been unexplored. The recent development of methods for optical recording of deep brain calcium dynamics has made it possible to measure for the first time how these neurons are regulated during behavior[16,19] (Figure 2).

Anticipatory control of thirst satiation

The SFO was the first structure to be examined[16]. Optical recording of calcium dynamics in SFO^{GLUT} neurons of freely behaving mice confirmed that these cells monitor changes in plasma osmolality, volume, and pressure, consistent with classic models[10-12]. Unexpectedly, these recordings also revealed that SFO^{GLUT} neurons are rapidly inhibited when thirsty mice begin to drink[16]. This rapid feedback is triggered by the detection of water in the oral cavity and is time-locked to the act of licking, enabling SFO^{GLUT} neurons to closely track the amount of water ingested. This suggests that SFO^{GLUT} neurons control drinking by making a comparison between two parameters: the level of physiologic need, which they measure by monitoring the blood, and the amount of water recently ingested, which they measure by tracking signals from the oropharynx. This model explains how drinking can rapidly quench thirst[55-58], yet nonetheless be properly metered to match an animal's physiologic deficit.

How water ingestion is detected in the oral cavity and then communicated to SFO^{GLUT} neurons is not well understood. One mechanism for water detection appears to involve temperature sensing in the oral cavity, as ingestion of cold water inhibits SFO^{GLUT} neurons more efficiently than ingestion of warm water and, indeed, isolated oral cooling (using cold, dry metal) can transiently inhibit SFO^{GLUT} neurons[16]. This finding suggests a neural basis for the everyday experience that cold liquids are experienced as more thirst-quenching[65-70] and raises the possibility that sensory fibers innervating the mouth that express the cold-activated channel transient receptor potential menthol type 8 (TRPM8)[71,72] participate in the detection of water.

Anticipatory control of vasopressin secretion

The PVH and SON are important downstream targets of the LT that control release of AVP into the circulation and thereby modulate water retention by the kidneys. Classic studies showed that the AVP concentration in the blood rapidly falls during drinking[76-79], but little has been reported about the *in vivo* dynamics of AVP neurons[77,80]. Recently, recordings of both calcium dynamics and electrophysiological activity in freely behaving animals demonstrated that AVP neurons in the PVH and SON are rapidly inhibited during drinking such that their activity returns to baseline before any change in the blood can occur[19]. Presystemic inhibition of AVP neurons appears to occur in two parts: a first phase in which these neurons are transiently inhibited by water-predicting cues alone, and a second phase in which they are durably inhibited by drinking. This second phase is likely mediated by inhibition of upstream SFO^{GLUT} neurons[16]. The rapid inhibition of AVP neurons explains why circulating AVP levels begin to decline at the outset of drinking in anticipation of the restoration of homeostasis.

Interactions with feeding

Many animals, including humans[81] and rodents[82-84], tightly coordinate eating and drinking to ensure that adequate water is available for food ingestion[83-86] and digestion[87] and also to counteract increases in plasma osmolality due to the absorption of osmolytes in food[88]. This stimulation of drinking by eating is known as prandial thirst. Conversely, dehydration potently suppresses food intake when water is unavailable[82,89-91], known as dehydration anorexia. Recent experiments have begun to reveal how these interactions between eating and drinking are controlled by the brain[16,19] (Figure 3).

Prandial thirst

Eating stimulates thirst within seconds, yet ingested food does not begin to alter the composition of the blood for several minutes. This raises the question of how the brain generates thirst at the outset of a meal. Optical recordings of SFO^{GLUT} neuron activity in hungry mice revealed that these cells are progressive activated by eating in a manner that tracks the amount of food consumed and precedes any change in blood osmolality[16], suggesting that this activation is driven by a signal originating from the oral cavity or early gastrointestinal tract that anticipates impending changes in the blood. Importantly, blocking this rapid activation by optogenetic silencing of SFO^{GLUT} neurons is sufficient to abolish meal-associated drinking. Thus, SFO^{GLUT} neurons are activated by a presystemic signal generated during eating, and this appears to be a primary cause of prandial thirst. The nature of the signal that communicates information about food ingestion to SFO^{GLUT} neurons is unknown and may involve both neural[83-86] and endocrine[92-103] components.

In addition to generating thirst, eating also triggers AVP secretion to promote renal water retention. Optical recordings of SON^{AVP} neurons during feeding revealed that these cells are also rapidly activated by eating[19], with kinetics very similar to those observed in upstream SFO^{GLUT} neurons[16]. This suggests that the presystemic signal communicated to the LT during eating controls both prandial thirst and AVP secretion.

Dehydration anorexia

The discovery that SFO^{GLUT} neurons are persistently activated by eating when water is unavailable[16] suggests that activation of these cells may also underlie dehydration anorexia. Indeed, optogenetic silencing of SFO^{GLUT} neurons during feeding eliminates the drive for prandial drinking and restores food intake to normal levels when water is unavailable[16], confirming that activation of SFO^{GLUT} neurons is necessary for the suppression in food intake caused by dehydration. The neural pathway through which SFO^{GLUT} neurons modulate the feeding circuit is unknown, but one possible downstream target is the PVH, a convergence point for signals that regulate energy and fluid balance.

Circadian regulation

Most drinking occurs during subjective daytime when animals are most active[104], and certain aspects of drinking behavior appear to be controlled directly by the brain's “central clock”, the suprachiasmatic nucleus (SCN)[107]. It was recently reported that vasopressinergic SCN neurons (SCN^{AVP} neurons) that project to the OVLT in mice are more active in the hours immediately preceding sleep, and that this increase in vasopressinergic neurotransmission can stimulate thirst by activating downstream OVLT neurons[18]. This increase in presomnial drinking prevents small (1–2%) increases and decreases in plasma osmolality and volume, respectively, that would otherwise occur during sleep[18]. SCN^{AVP} neurons also project directly to the SON, and this projection has been proposed to facilitate an increase in OVLT-stimulated AVP secretion by SON^{AVP} neurons during late sleep[108]. Complementary control of the thirst circuit by the SCN may prevent nighttime dehydration by stimulating water intake before sleep and, later, by promoting water retention during sleep.

Salt appetite

Sodium is a major determinant of plasma osmolality, and dehydrated animals therefore must consume both water and sodium in order to fully restore fluid homeostasis. The motivational drive to consume sodium, known as salt appetite (reviewed by ref. 109), is likely controlled by genetically hard-wired neural circuits, but the organization of these circuits has been unclear.

One important signal of sodium deficiency is the hormone aldosterone[110,111], which acts both at the kidneys to promote sodium conservation[112,113] and in the brain to stimulate salt appetite[114,115]. Aldosterone can activate the mineralocorticoid receptor (MR) only in cells that express the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD2)[116], and co-expression of MR and HSD2 in the brain is restricted to a small population of neurons in the nucleus of the solitary tract (NTS^{HSD2} neurons)[117]. This unique population of aldosterone-sensitive neurons is specifically activated during sodium deprivation[118], and recent chemogenetic manipulations demonstrated that activation of NTS^{HSD2} neurons is both necessary and sufficient to drive sodium consumption[20]. Projections from NTS^{HSD2} neurons are restricted to three brain regions that previously have been implicated in the control of salt appetite[20,119,120]: to *Foxp2*-expressing neurons in the pre-locus coeruleus and lateral parabrachial nucleus, and to an unidentified population of neurons in the BNSTvl.

Interestingly, an independent study recently showed that optogenetic stimulation of the SFO^{GLUT}→BNSTvl projection also drives sodium consumption[21], identifying the BNSTvl as a convergence point for the neural control of salt appetite.

Open Questions

Despite recent progress, many fundamental questions about the thirst circuit remain unresolved. Here we highlight some of the key unanswered questions.

How is water in the oral cavity detected and signaled?

The discovery that the LT is potently modulated by signals from the oral cavity during eating and drinking[16] reemphasizes the importance of understanding how food and water are detected in the mouth. Yet, at present little is known about the specific molecules and cell types that detect oral signals relevant to fluid balance. In *Drosophila*, water detection is mediated by a discrete population of gustatory neurons expressing the water taste receptor pickpocket protein 28 (PPK28)[121], but it is unclear whether water constitutes a distinct taste modality in mammals[122]. It is likewise unclear how the sensations of osmolality, temperature, dryness, and pressure are detected in the oropharynx and early gastrointestinal tract and then communicated to the LT.

What are the molecular identities of the osmosensor and baroreceptor?

Plasma osmolality and pressure are thought to be detected by mechanosensitive ion channels expressed within osmosensitive and pressure-sensitive neurons, but the identity of these channels has remained elusive. While a number of candidates have been advanced[123-130], it remains controversial whether any of these proteins function as direct sensors. For example, while early studies suggested a role for the cation channels transient receptor potential vanilloid type 1 (TRPV1)[123,124] and type 4 (TRPV4)[125,126] in osmosensation, later work found that rodents lacking these genes display normal regulation of drinking and fluid balance[131-133], implying that other molecules are involved. Similarly, while acid-sensing ion channel 2 (ASIC2) appears to play a role in blood pressure sensing by peripheral nerves innervating the aortic arch and carotid sinus[129,130], it may not be the mechanosensitive "baroreceptor" that directly detects changes in pressure. The ability to access molecularly-defined populations of interoceptive neurons will likely provide new insights into these questions.

How does thirst engage learning, motivation, and motor systems?

Thirst is one of the most potent motivational drives in mammals, yet it remains almost completely unknown how thirst neurons in the LT connect to brain regions important for reinforcement and learning[134]; how this input generates a motivational drive that is specific for water consumption relative to competing homeostatic needs; and how conflicts between various homeostatic drives are resolved in the brain. It is similarly unclear how changes in LT neuron activity are communicated to motor pattern generators in the brainstem that control licking and swallowing[135,136], which are ultimately the sites in the brain where drinking behavior is controlled.

Implications and conclusions

Genetic tools for circuit analysis have reinvigorated the study of thirst[13-21], enabling new insight into the cells, pathways, and dynamics that underlie drinking behavior. While much remains to be discovered, one sign of recent progress is the emergence of circuit mechanisms that can potentially explain elements of everyday human experience, including prandial and circadian thirst; dehydration anorexia; rapid thirst quenching; and the effects of oral cooling. While none of these phenomena is yet understood in detail, we now have a foothold at the level of neural circuits, which can serve as a starting point for further investigation. We expect rapid progress toward understanding these and other aspects of drinking behavior over the next few years.

Acknowledgments

The authors thank members of the Knight lab for comments and discussion. C.A.Z. acknowledges support from the Genentech Foundation Predoctoral Fellowship, UCSF Discovery Fellowship, and NSF Graduate Research Fellowship (1144247). D.E.L. acknowledges support from the ARCS Foundation Scholarship, UCSF Discovery Fellowship, and the NIH (F31-HL131463). Z.A.K. is a New York Stem Cell Foundation-Robertson Investigator. Z.A.K. acknowledges support from the New York Stem Cell Foundation, the American Diabetes Association Pathway Program, the Rita Allen Foundation, the McKnight Foundation, the Alfred P. Sloan Foundation, the Brain and Behavior Research Foundation, the Esther A. & Joseph Klingenstein Foundation, the Program for Breakthrough Biological Research, and the UCSF DERC (P30-DK06372) and NORC (P30-DK098722). This work was supported by an NIH New Innovator Award (DP2-DK109533), R01-NS094781, and R01-DK106399.

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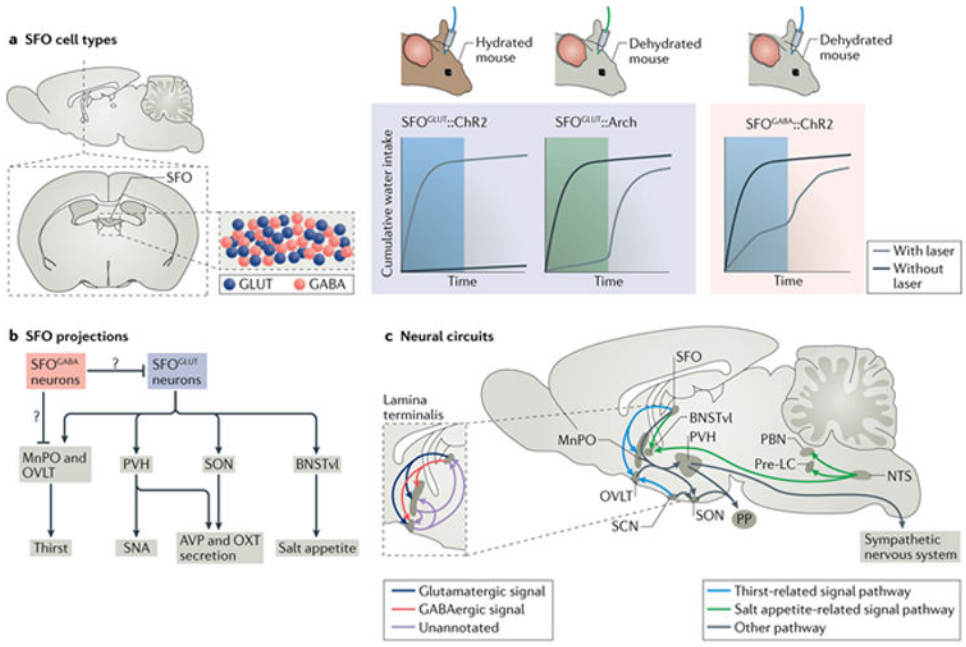

Figure 1. Structure of the neural circuits underlying thirst and fluid homeostasis in mammals

a | The SFO is situated immediately dorsal to the third ventricle and contains intermingled populations of glutamatergic (SFO^{GLUT}) and GABAergic (SFO^{GABA}) neurons with opposing effects on drinking behavior. Optogenetic activation of SFO^{GLUT} neurons (blue area, laser on, 30 min) stimulates intense drinking in hydrated mice, whereas optogenetic silencing of SFO^{GLUT} neurons (green area, laser on, 15 min) suppresses drinking in dehydrated mice. In contrast, optogenetic activation of SFO^{GABA} neurons (blue area, laser on, 15 min) suppresses drinking in dehydrated mice. **b** | SFO neurons innervate several brain regions involved in the regulation of fluid balance. SFO^{GLUT}→MnPO/OVLT projections drive thirst, whereas the SFO^{GLUT}→BNSTvl projection promotes sodium consumption. SFO^{GLUT}→PVH/SON projections have not yet been functionally annotated with cell type-specificity, but classic models suggest that SFO^{GLUT}→PVH/SON projections mediate secretion of AVP and OXT into the circulation and that the SFO^{GLUT}→PVH projection may also modulate SNA and thereby alter blood pressure and heart rate. However, projections from MnPO/OVLT neurons to the PVH/SON also likely contribute to these neuroendocrine and autonomic responses. Projections from SFO^{GABA} neurons to the MnPO/OVLT, as well as locally within the SFO, have not yet been functionally annotated. **c** | Sagittal illustration of the cell type-specific neural circuits underlying thirst and fluid homeostasis in the mouse brain. The LT consists of two circumventricular organs (SFO and OVLT) and an integratory structure (MnPO). Information about plasma osmolality, volume, and pressure enters the LT through specialized interoceptive neurons in the SFO and OVLT, some of which are intrinsically osmosensitive and AngII-sensitive (e.g., SFO^{GLUT} neurons). The LT nuclei communicate with each other through an extensive network of bidirectional projections that has not yet been fully mapped with cell type-specificity. Outside the LT, SFO^{GLUT} neurons project to the PVH, SON, and BNSTvl; projections from the MnPO and OVLT have not yet been mapped with cell type-specificity, however projections from these regions to the PVH and SON are well-established. SCN^{AVP} neurons project to the OVLT and SON to mediate

circadian regulation of thirst and AVP secretion, respectively. Information about plasma sodium enters the circuit through specialized aldosterone-sensitive NTS^{HSD2} neurons, which promote salt appetite and project to the pre-LC, PBN, and BNSTvl. Arch, archaerhodopsin; AVP, vasopressin; BNSTvl, ventrolateral part of the bed nucleus of the stria terminalis; ChR2, channelrhodopsin-2; MnPO, median preoptic nucleus; NTS, nucleus of the solitary tract; OVLT, organum vasculosum of the lamina terminalis; OXT, oxytocin; PBN, parabrachial nucleus; PP, posterior pituitary; pre-LC, pre-locus coeruleus; PVH, paraventricular hypothalamus; SCN, suprachiasmatic nucleus; SFO, subfornical organ; SNA, sympathetic nerve activity; SON, supraoptic nucleus.

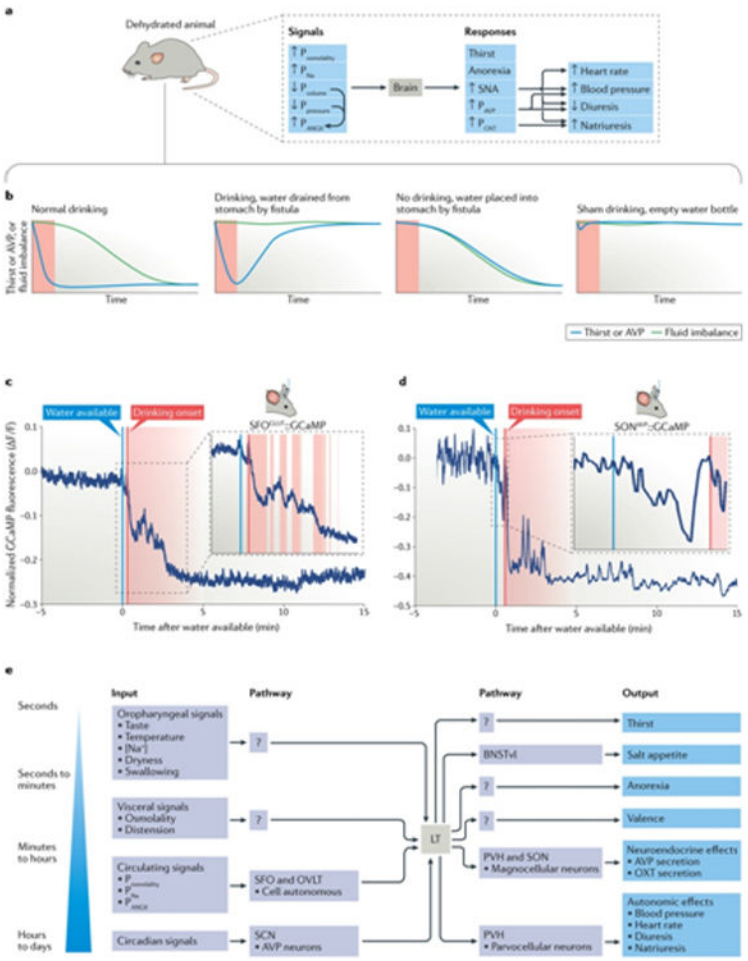

Figure 2. Anticipatory and homeostatic regulation of the thirst circuit

a | Challenges to fluid homeostasis, such as water deprivation, cause deviations in the composition of the blood. These deviations can include increases in plasma osmolality ($P_{osmolality}$) and sodium (P_{Na}), as well as decreases in plasma volume (P_{volume}) and pressure that stimulate renin secretion by the kidneys and, consequently, AngII production (P_{AngII}) in the blood[137]. These homeostatic circulating signals are translated by the brain into counter-regulatory responses, including thirst, anorexia, vasopressin (P_{AVP}) and oxytocin (P_{OXT}) secretion, and sympathetic nerve activation (SNA). While thirst is ultimately necessary to restore fluid homeostasis, this coordinated set of responses also promotes water retention (antidiuresis) and sodium excretion (natriuresis) by the kidneys, suppresses ingestion of food (and, therefore, sodium and other osmolytes), and modulates blood pressure and heart rate in order to maintain fluid homeostasis until water can be ingested. **b** | When thirsty animals are allowed to drink (red area), thirst and AVP secretion (blue lines) are rapidly inhibited before the composition of the blood (green lines) is corrected by ingested water. Historical experiments using esophageal/gastric fistulae and sham drinking suggest that this anticipatory regulation involves both immediate oropharyngeal and delayed visceral signals, however the neural mechanism underlying rapid anticipatory regulation of thirst and AVP secretion remained unexplored until recently. **c** | Fiber photometry recordings revealed that

SFO^{GLUT} neurons are rapidly inhibited during drinking to coordinate the anticipatory control of thirst and AVP secretion[16]. In this example recording, a thirsty mouse (48 h water restriction) is given access to water. The inset highlights that rapid inhibition of SFO^{GLUT} neurons is time-locked to individual drinking bouts (red areas). **d** | Fiber photometry recordings confirmed that SON^{AVP} neurons are also rapidly inhibited during drinking[19]. In this example recording, a thirsty mouse (24 h water restriction) is given access to water. The inset highlights that SON^{AVP} neurons are transiently inhibited by water-predicting cues *before* drinking is initiated and that this inhibition is rapidly reset in the seconds immediately prior to water ingestion. The neural mechanism and physiological significance of this transient pre-ingestive inhibition remain unclear. **e** | In addition to homeostatic circulating signals (e.g., $P_{osmolality}$, P_{Na}, and P_{AngII}) that were canonically thought to activate the LT (see **Panel a**), recent experiments have revealed that a diverse set of anticipatory oropharyngeal, visceral, and circadian signals also influence SFO^{GLUT} neurons and the LT on different time-scales. This convergence of homeostatic and anticipatory signals allows SFO^{GLUT} neurons and the LT to control a diverse set of behavioral, neuroendocrine, and autonomic outputs both *in response to* and *in anticipation of* deviations from fluid homeostasis. Data in Panel 2c is adapted from ref. 16. Data in Panel 2d is adapted from ref. 19.

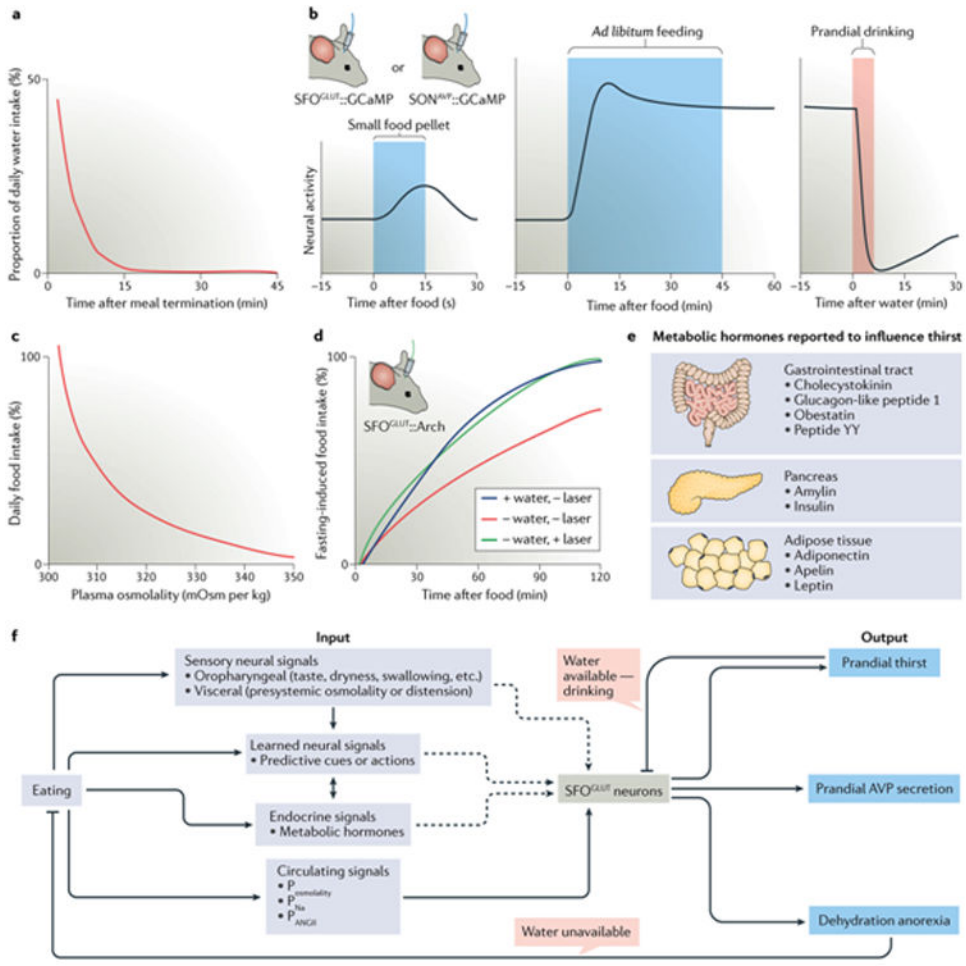

Figure 3. The thirst circuit monitors and controls feeding behavior

a | Eating potently stimulates prandial thirst, and most drinking therefore occurs in close temporal proximity to eating. Schematic is based on data in ref. 84. **b** | SFO^{GLUT} neurons and SON^{AVP} neurons are rapidly activated on both short (seconds) and long (minutes) time-scales during feeding to promote prandial thirst and AVP secretion. Schematics are based on data in refs. 16,19. **c** | Dehydration potently suppresses food intake, and increases in plasma osmolality therefore inhibit feeding. Schematic is based on data in ref. 138. **d** | Dehydration anorexia causes hungry mice (24 h food restriction) to consume less food when water is absent (red line) than when water is available (black line). However, this suppression of food intake is completely alleviated by optogenetic silencing of SFO^{GLUT} neurons during feeding (green line), indicating that activation of SFO^{GLUT} neurons promotes dehydration anorexia in addition to prandial thirst. Schematic is based on data in ref. 16. **e** | Metabolic hormones secreted during feeding by the gastrointestinal tract[92-95], pancreas[96-100], and adipose tissue[101-103] have been proposed to regulate the electrical activity of SFO neurons *ex vivo*. However, it remains unclear whether such endocrine signals contribute to the natural coordination of eating and drinking by SFO^{GLUT} neurons *in vivo*. **f** | Eating rapidly activates SFO^{GLUT} neurons in order to promote prandial thirst and AVP secretion as well as

dehydration anorexia. The mechanism of this activation remains unclear, but may involve learned or sensory neural signals or endocrine signals.

Table 1

Cell type-specific manipulations in the thirst circuit

Pathway	Genetic marker	Manipulation	Neural activity	Water intake	Food intake	Sodium intake
SFO^{GLUT} somas	*Camk2*[13,16], *Etv1*[13], *Nos1*[14,16]	ChR2	↑	↑[13,14,16]	_ [13,14,16]	↑[13]
SFO^{GLUT} somas	*Camk2* [15], *Nos1* [14]	G_q-DREADDs	↑	↑[14,15]	_ [14]	↑[15]
SFO^{GLUT} somas	*Nos1*[16]	Arch	↓	↓	↑	N.R.
SFO^{GLUT}→MnPO	*Camk2* [16]	ChR2	↑	↑	N.R.	N.R.
SFO^{GLUT}→PVH	*Camk2* [16]	ChR2	↑	–	N.R.	N.R.
SFO^{GLUT}→OVLT	*Slc17a6* [21]	ChR2	↑	↑	N.R.	–
$SFO^{GABA+GLUT}$→OVLT	–[21]	Arch	↓	↓	N.R.	–
SFO^{GLUT}→BNSTvl	*Slc17a6* [21]	ChR2	↑	–	N.R.	↑
SFO^{GLUT}→BNSTvl	–[21]	Arch	↓	–	N.R.	↓
SFO^{GABA} somas	*Slc32a1*[13,21]	ChR2	↑	↓[13,21]	_ [13]	_ [13], ↓[21]
MnPO/$OVLT^{GLUT}$ somas	*Slc17a6*[17]	ChR2	↑	↑	N.R.	N.R.
MnPO/$OVLT^{GABA}$ somas	*Slc17a6*[17]	ChR2	↑	↓	–	N.R.
SCN^{AVP}→OVLT	*Avp*[18]	ChR2	↑	↑	N.R.	N.R.
SCN^{AVP}→OVLT	*Avp*[18]	Arch	↓	↓	N.R.	N.R.
NTS^{HSD2} somas	*Hsd11b2*[20]	G_q-DREADDs	↑	–	↓	↑
NTS^{HSD2} somas	*Hsd11b2*[20]	G_i-DREADDs	↓	–	N.R.	↓
NTS^{HSD2} somas	*Hsd11b2*[20]	TetTox	↓	Fatal (>20% body weight lost byday 10)		